

Effect of grapes' color on the microbial activity of fresh and preserved grapes

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ABSTRACT:

There are two objectives for this study. First is to identify the microbial growth on fresh grapes and compare it with that on preserved ones. Second is to study the effect of grapes' color on the microbial growth. All samples were placed in an appropriate environment to the microbial growth for 10 days. The procedures were repeated five times in order to get pure cultures. It was found that different colors of fresh grapes had different microorganisms and even different species from the same genus depending on the amount of antioxidants that each color contained. Preserved grapes had fewer microorganisms compared to the fresh ones due to their high sugar content and low water activity. Since preserved grapes contain high sugar content, no bacterial growth was predicted. There is an inverse proportion between the amount of antioxidants and number of microorganisms a fruit has.

Keywords: Grapes, Microbial, fresh, preserved

INTRODUCTION

Grapes are very delicate and smooth-skinned fruit. The fruit is a berry borne in what are called bunches. Scientifically, they are named *Vitis vinifera*, *Vitacea* [1]. Their pH is 3.4-4.5 and considered as a low acid fruit. Grapes mainly consist of 81.9% of water besides many other nutrients like carbohydrates (14.9%), protein (1.4%), fat (0.4%), minerals and vitamins (0.4%). In addition, they contain important phytonutrients include flavonoids such as anthocyanins; which are responsible for producing the color in red grapes, and other polyphenolic compounds like resveratrol, lycopene and beta-carotene [2].

On the basis of nutrient content of grapes, they would appear to be capable of supporting the growth of bacteria, yeasts, and molds which are the main cause of microbial spoilage, hence they considered as one of the potential hazardous food (PHF). Despite the high water activity of grapes, their low pH that is below the level that generally favors bacterial growth leads to their spoilage being dominated by fungi. This one fact alone would seem to be sufficient to explain the absence of bacteria in the grape spoilage [3].

During harvesting, processing and handling operations grapes may become contaminated with a wide range of microorganisms. Consequently, during distribution and storage period the conditions will be favorable for certain organisms to multiply and spoil the grapes. In general, microbial spoilage of grape involves any change that renders the grape unacceptable for human consumption. Yeast and mould are more tolerant of low water activity and low pH than bacteria and they typically spoil fruits. Fungi produce pectinolytic enzymes which soften the plant tissues causing rot. As much as 30% of all fruit spoilage may be due to

Penicillium [4]. *Botrytis cinerea* is by far the most serious cause of spoilage in table grapes [5]. Other fungi spoiling grapes are *Cladosporium spp.* causes black rot, *Penicillium spp.* causes blue mould, and *Rhizopus stolonifer* causes watery soft rot [6]. *Penicillium* species do not usually attacks grapes before harvesting, but are common in stored grapes [7].

One way of reducing the hazards associated with grape spoilage is to prevent or slow down microbial growth which is itself dependent on a number of factors such as the nutritional content of the food, temperature, pH, presence of inhibitors, and water activity. All these factors are exploited by food microbiologists for food preservation. Many food preservation processes have been developed to deal with this problem. The main aim of food preservation is to minimize the growth of microorganisms during the storage period, thus promoting longer shelf-life of the product. The most common preservation methods are freezing, pasteurizing, canning, drying, salting and sugaring. Grapes can be preserved by different preservation processes. Such processes that concern us in this experiment are drying, sugaring, and pasteurizing [3].

The water availability is considered as the major factor in controlling food spoilage [8]. Both the drying temperature and the decreased water activity (a_w) affect microbes during the drying process. The intended effect of grapes' drying is to halt the growth of all microorganisms when a_w is lower than 0.6. Raisins have 0.6-0.65 water activity which is usually much lower than the microbial growth minimum, therefore, raisins are microbiologically stable [8]. In order to preserve grape juice, pasteurization process must be performed. Pasteurization is the term given to heating

processes typically in the range 60-80°C and applied for up to a few minutes [9]. In fact, heat is the most widely used method for killing microbes. Pasteurizing grape juice aims to eliminate a large proportion of potential spoilage organisms, thus extending its shelf-life. Because of this, pasteurized fruit juices take longer to spoil, especially when they are refrigerated to delay the growth of surviving organisms [8]. Since sugar is a hygroscopic compound, it can preserve food by lowering its water activity. For that reason, sugaring plays a major role in preventing food spoilage. Grapes can be preserved by sugaring which is done by cooking the grape in sugar to the point of crystallization and the resultant product, jam, is then stored dry. Cooking grapes in high sucrose concentration create too high osmotic pressure for most microbial survival as well as lower the water activity. The intent of adding sugar is to stop microbial growth when a_w is less than 0.7 [10].

There had been two objectives for this experiment. First was to identify the microorganisms that cause grape spoilage, and compare them with microorganisms that cause spoilage on preserved ones. Second was to study the effect of grapes' color on the microbial activities.

MATERIALS AND METHODS

Preparation of Samples

Fresh grape, grape jam, grape juice, and raisins were purchased, and placed in sterile plastic boxes in an appropriate environment for microbial growth (high temperature, dark, moist, and tightly closed boxes) for 10 days. In order to study whether the color of grapes itself affects the microbial growth; three common colors of fresh grapes were chosen (red, green, and black).

Microbiological analysis

Sub-culturing and Spreading

Subculturing was done under aseptic technique for all samples (fresh grape, jam, raisins, and juice). An inoculum was taken from each kind of microorganism from each sample and introduced on Sabourand's dextrose ager (SDA) to determine fungal species. The spreading method was performed for the liquid that resulted from spoiled fresh grapes. By using a sterilized pipette, 0.1 mL of the spoiled grape liquid was transferred to a plate count ager (PCA) to determine bacterial species. The transferred amount of the sample was spread by the sterilized glass rod (with 95% ethyl alcohol) over the ager surface.

Incubation and four-way streak method

All SDA plates were incubated at 25°C for 7 days and the PCA plates were incubated at 30°C for 2 days. For

bacterial growth, four-way streak method was used for the most dominant kind of bacteria that have been grown on PCA plates in order to get an isolated colony. After that, the plates were incubated at 25°C for 48-72 hours. After incubation, the first results were mixed cultures so subculturing was repeated five times in order to get pure cultures and to avoid any contamination.

Cultural characteristics and microscopic identification

Cultural characteristics and microscopic identification were performed for fungal and bacterial growth in order to identify them. Texture, back and front color, and colonial topography were reported for each kind of fungi in all SDA plates. On the other hand, form, size, color, elevation, and edge of the colonies were reported for each kind of bacteria on all PCA plates. Then, fungal staining was done for all fungi species that were found in all samples by using lactophenol cotton blue stain. For bacteria, Gram staining was done for the most dominant colonies by using crystal violet, Gram's iodine mordant, and Safranin stains.

RESULTS AND DISCUSSIONS

After incubation, different colors of fresh grapes resulted in different microbial growth. Red ones had one kind of Gram negative bacteria that were found in the liquid (Table.1) and one kind of fungi which was *Asperigillus niger*. While black ones had an only fungal growth (*Asperigillus niger*, *Penicillium chrysogenum*, and *Penicillium funiculosum*). Similarly, green grapes had an only fungal growth (*Asperigillus niger*, *Penicillium aurantiogrisum*, and *Penicillium purpurogenum*) (Table.2). Grape jam had three types of fungi; *Asperigillus niger*, *Eurotium*, and *Penicillium citrinum*. Raisins showed only one kind of fungi (*Asperigillus niger*) which was the most common one almost in all samples. Grape juice had only one type of fungi which was *Muscodor albus* (Table.3).

Table 1 reports the bacterium that was found in the liquid of spoiled red grapes. At the microscopic level, it was hard to identify the name of bacteria, however from the cultural characteristics it was Gram negative bacteria with bacillus, single and pink color cells. Since bacteria cannot tolerate high acidity of grapes [3], the presence of bacteria was in the spoiled liquid.

Table 2 reports the microbial growth on red, black, and green grapes samples. *Aspergillus niger* was the most dominant fungi that found on all samples of fresh grapes. This fungus had cottony texture on Sabourand's dextrose ager (SDA), and its colony was consisted of a solid yellowish basal covered by a dense layer of dark brown to black conidial heads. According to P. Mikusova et al, (2009) the most isolates of black

Aspergillus strains that have been found on grapes belong to the species *A. niger*. Red grapes showed only *A. niger* while black and green ones showed different *Penicillium spp* as well as *A. niger*. *Penicillium spp* that were found on black grapes were *P. chrysogenum* and *P. funiculosum*, these species were had granular texture on SDA with green front color and their colonies were umbonate in orange back color. Nevertheless, other different species of *Penicillium* were found on green grapes. The colonies of *P. aurantiogrisum* were in a velvety texture with green front color and rugose compact basal in yellowish color. The colonies of *P. purpurogenum* were had granular texture with dark green front color and umbonate basal in red color.

Table 1: Unknown bacterium that was found in the liquid of spoiled red grapes as determined through the use of shaker/stomacher homogenization then plated on Acidified PDA and TSA and incubated at 30°C

Sample	Microscopic Identification	Average Count (CFU/orange) ^c
Red grapes	Gram negative bacteria with pink color, bacillus and single cells.	Form: circular, Size: pinpoint, Elevation: raised, Color: creamy, Edge: entire

Table 2. Cultural characteristics of samples taken from the three types of fresh grapes tested and resulting presumptive microorganism

Sample	Cultural Characteristics	Presumptive Microorganism
Red grape	Cottony, Black, Rugose	<i>Aspergillus niger</i>
Green grape	Velvety, Green, Rugose	<i>Penicillium aurantiogrisum</i>
	Cottony, Black, Rugose	<i>Aspergillus niger</i>
	Granular, green, Umbonate	<i>Penicillium purpurogenum</i>
Black grape	Cottony, Black, Rugose	<i>Aspergillus niger</i>
	Granular, Green, Umbonate	<i>Penicillium chrysogenum</i>
	Granular, Orange, Umbonate	<i>Penicillium funiculosum</i>

Table 3. Cultural characteristics of samples taken from on preserved grapes and resulting presumptive microorganism

Sample	Cultural Characteristics	Presumptive Microorganism
Raisins	Cottony, Black, Rugose	<i>Aspergillus niger</i>
Jam	Velvety, Green, Umbonate	<i>Eurotium</i>
	Cottony, Black, Rugose	<i>Aspergillus niger</i>
	Velvety, green, Rugose	<i>Penicillium citrinum</i>
Grape juice	Cottony, Black, Rugose	<i>Aspergillus niger</i>

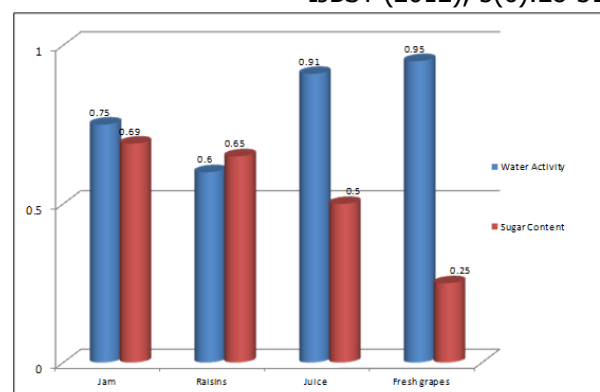


Figure 1. Sugar content and water activity of preserved and grapes

The identification of *Penicillium* isolates to the species was done by comparing their microscopic morphology, colony diameters, colors of conidia and colony pigments with the literature. For example, the colonial and cellular morphology of *P. purpurogenum* that was found on green grapes was identified by the microscopic identification and compared with the literature.

When comparing between the microorganisms that were found on different colors of grapes, the difference and the variation of fungal growth was due to high concentration of the antioxidant compounds, anthocyanins; which were responsible for producing the dark color in red and black grapes according to Meyer et al. (1997). In fact, the darker the color of grapes the highest concentration of antioxidants would be. These antioxidants prevent the growth of the most fungal species. For that reason, there was a less fungal growth on red grapes than green and black grapes. However, black grapes were dark in color but the presence of fungal growth was found on the bunch of grapes.

Table 3 reports fungal species that were found on different preserved grapes. Both jam and raisin samples have had *A. niger* growth. In addition, there was growth of *Eurotium* (the *A.glaucus* series) and *P. citrinum* in the jam sample. *Eurotium* colonies had a velvety texture with yellowish green front color and umbonate basal in brownish yellow color. Each colony of *P. citrinum* was in a velvety texture with green front color and rugose compact basal in yellowish color. The colonies of *Muscodora albus* that was found on the grape juice sample were in a velvety texture with white crystal front color and rugose compact basal in yellowish color. According to Smilanick et al. (2010), *Muscodora albus* can combat *Botrytis cinerea* which is the main cause of gray mold in table grapes. Furthermore, *Muscodora albus*, an endophytic fungus, produces a mixture of volatile organic compounds that are harmless to people and animals. These compounds

can kill or inhibit the spread of certain other microbes on grapes, such as *B. cinerea* [14].

Different preserved samples resulted in different microbial growth. Given that bacteria cannot resist high sugar content and low water activity, no bacterial growth was predicted. The difference of fungal growth in preserved samples was due to the high concentration of sugar content. Figure 1 demonstrates the sugar content and water activity of all samples. *Penecillum spp.*, *Eurotium spp.* and *Asperigellius niger* were capable of growth below water activity 0.85 and resist high sugar content as raisin had the lowest a_w value which was 0.6 and jam had lowest sugar content (0.5) [15]. High sugar content in preserved grapes had minimized the microbial growth by lowering the water activity.

Mainly, the variation of the fungal growth in both fresh and preserved grapes was due to the water activity value and sugar content. More fungal growth was shown on fresh grapes compared to the preserved ones, thus was due to high water activity of fresh grapes (0.95) that supports the microbial growth. In contrast, preserved grapes had low water activity and contained high sugar content which minimized the microbial growth, especially the bacteria. Sugar had been able to lower the water activity because it's a hygroscopic compound that has high binding capacity with water molecules.

In conclusion, different types of fresh grapes contain mostly the same nutrients but differ in the amount of antioxidants (flavonoids) which lead to the growth of different spoilage microorganisms. Results showed that spoiled preserved grapes have fewer microbial growth compared to fresh ones, thus indicates that food preservation processes do indeed prevent and/or reduce microbial food spoilage. The dehydration process gave the best results in preserving the grapes from spoilage in spite of its simplicity.

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